Please review following topics:

Site-specific fragmentation of DNA

 In nature there found many different enzymes called restrictases or more properly restriction endonucleases. These enzymes recognize specific short sequences within target DNA molecules. If that sequence is found the restrictase will cut the DNA molecule by introducig breaks (nicks) into both strands of DNA



Hundreds of different restrictases recognizing unique DNA sequences were discovered. They are widely used for DNA analysis and engineering.

Separation of DNA

- DNA molecules could be separated by size using gel electrophoresis
- DNA molecules are negatively charged thus if electric field is applied to DNA solution the molecules will migrate to the positive electrode. This movement of molecules in electric field is called electrophoresis.

- The speed of the movement depends on the ratio of charge of the molecule to its molecular mass.
- DNA is a periodic molecule thus the charge to molecular mass ratio is approximately the same for all DNA molecules
- Thus, if there is no resistance on the way all DNA molecules would migrate with the same speed
- In order to separate DNA molecules by size the electrophoresis is performed in a gel a special porous medium. The size of the pores is comparable to the separating DNA molecules' size.
- Larger DNA molecules will encounter stronger resistance and migrate slower than smaller DNA molecules.



- DNA is visualized with special dyes that bind to it. Most commonly used is Ethidium Bromide – a fluorescent dye.
- DNA fragment size is determined by comparing the position of the fragment on the gel to the molecular weight marker.





- Staining with ethidium bromide or other fluorescent dyes allows to detect all DNA in the gel
- In some experiments DNA molecules with specific sequence need to be detected
- Commonly used method Southern blotting and hybridization with labeled sequence-specific probe

Characterization: Southern blot hybridization



-transfer of DNA from a gel to a membrane (e.g., nitrocellulose, nylon) -developed by Edwin Southern





